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RESEARCH ARTICLE

Animal Model of Pancytopenia and Bone Marrow Function Diagnosis of Domestic Rabbits

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Abstract

The present research aims at showing the effects of extracts of some plants seeds (Nigella sativa, Medicago sativa, Eruca sativa, Ocimum basilicum, Vitis vinifera, Portulaca oleracea) in animals that are induced by a type of drug Azathioprine as animal model of pancytopenia. Several reports have shown that plant extracts are used as an alternative to chemotherapy in many cases of diseases. The research was conducted on forty white rabbit (five weeks old), after weaning, weighing between 500-700 grams, the method of work included eight groups, the numbers of blood cell types that indicate the effectiveness of bone marrow were measured (RBC, WBC, Thrombocytes, IRF, IPF,IGs, Schistocytes, reticulocyte and ANC),the extracts showed different effects in terms of their ability to improve or re-develop bone marrow to produce blood cells, Ocimum basilicum extract showed a higher effect than the rest of the extracts in pancytopenia treatment, which gave a clear effect on improving bone marrow efficiency.

Introduction

Pancytopenia is an important clinic hematological entity encountered in our day to-day clinical practice, it is not a disease by itself but a triad of haematological finding that can result from a number of disease processes [1]. It can be a feature of many serious and life threatening illnesses like drug induced bone marrow hypoplasia, fatal bone marrow aplasia, and leukaemias [2]. It can result from failure of production of stem cells in bone marrow, infiltration of bone marrow by malignant cells or fibrosis, immune mediated bone marrow suppression, ineffective erythropoiesis and dysplasia, peripheral sequestration of blood cells by overactive reticuloendothelial system, and immune or nonimmune mediated increased destruction of blood cells. Incidence of various disorders causing pancytopenia varies according to geographical distribution and genetic mutations [3].It is a hematological feature of varying a etiology whereas it is commonly associated with viral infection (HIV), Megaloblastic anemia due to nutritional deficiencies, hypersplenism (congestive splenomegaly), apla-stic anaemia, myelodysplastic syndrome, subleukaemic leukaemias, military tuberculosis, multiple myeloma, paroxysmal nocturnal haemoglobinuria, malignant infiltration, and drug induced pancytopenia.it is usually presents with the clinical sign and symptoms of bone marrow failure such as pallor, easy fatigability, dyspnoea, bleeding or bruising, and increased tendency to infection [4,5]. Azathioprine (AZA) is a cytotoxic immuneosup-pressive drug used in the prevention of rejection in organ transplants and the treatment of auto-immune diseases. However, AZA is haemotoxic causing significant bone marrow depression [6]. It is prodrug of 6-mercaptopurine (6-MP) syn-thesized by attaching an imidazole ring to the sulfur atom at the 6 position of the 6-MP molecule. The success in the field of kidney transplantation in 1962 broadened its clinical applications thereafter. This immunosuppressant is now widely used in dermatology, oncology, gastroenterology and rheumatology for its anti-leukemic, anti-inflammatory, and immunosuppressive properties [7, 8]. Current therapies for pancytopenia include bone marrow stimulant drugs, blood transfusion and bone marrow transplant, so the current therapies are very excruciating and have long-term side-effects.

Therefore, treating these condition using herbal drugs is very important, in the recent years significant attention has been directed towards exploring plant based natural organic compounds therapies, large number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids. The aim of this study was to investigate the therapeutic susceptibility of seed extracts of Nigella sativa, Medicago sativa, Eruca sativa, Ocimum basilicum, Vitis vinifera, Portulaca oleracea, in the treatment of pancytopenia of domestic rabbits.

Material and Methods

Experimental Animals

The research was conducted on forty white rabbit (five weeks old), after weaning, weighing between 500-700 grams, the method of work included eight groups, the animals were placed in the animal house of Faculty of Science, University of Kufa, with standard environment situations temperature (25-28 C°) and 12 hour light-dark cycle, The study protocol was done with agreement by the ethical committee of the Department of Biology- Faculty of Science-University of Kufa. The domestic rabbits kept in animal house for acclimation to the laboratory condition for one week before using them.

Drug of Induce

Azathioprine was used to induce pancytopenia in white rabbits were given orally by intra gastric intubation at dose 2 mg / kg / day for 45 days [9].

Estimation of Cells

The numbers of [WBCs, PLTs, RBCs, schistocytes, reticulocytes, IRF, IPF, and IGs] were estimated by a device (Sysmex XE) [10, 11]. ANC was also measured by a special equation by method [12].

Extraction of Plant

the seeds of plants that used in current experiment cleaned and dried, then these seeds were crushed by blender to produce powder, the extraction of oil from seeds done according to the method of Stahl[13]by addition 150 ml petroleum ether with 20 g of seeds powder and by using Soxhlation for 24 hour, 40-60 C°, and the solvent evaporated by using the rotary evaporator at 60 C° to complete the evaporation process of solvent, and the remaining oil were used for concentrations preparation, the emulsion was prepared by using dry gum method according to[14].This method is referred as 4:2:1 for every 4 portions by volume of oil, 2 portions of water and 1 portion of acacia are added in preparing the primary emulsion[15]. Firstly, Acacia was weighted and put into dried cleaned blender then measured amount of oil added to acacia and mixed homogenously until cracking sound produced. When the mixture became sticky, distilled water was added and mixed well to form emulsion which is creamy white, in a ratio of 4:2:1.

Study Protocol

The rabbits used in the experiment were divided by 5 rabbits per group as the following:

Group (1) Negative control group in which rabbits were given acacia by intra gastric intubation at dose 100 mg/kg (for 45 days).

Group (2) Positive control group in which rabbits were given of acacia by intra gastric intubation at dose 100 mg/kg and Azathioprine 2 mg / kg / day (for 45 days).

Group (3) experiment group were administrated of emulsion of seed extracts of Nigella sativa plant by intra gastric intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Group (4) experiment group were administrated of emulsion of seed extracts of Medicago sativa plant by intra gastric intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Group (5) experiment group were administrated of emulsion of seed extracts of Eruca sativa plant by intra gastric intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Group (6) experiment group were administrated of emulsion of seed extracts of Ocimum basilicum plant by intra gastric intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Group (7) experiment group were administrated of emulsion of seed extracts of Vitis vinifera plant by intra gastric intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Group (8) experiment group were administrated of emulsion of seed extracts of Portulaca oleracea plant by intra gastric

intubation at crud dose and Azathioprine 2 mg / kg / day (for 45 days).

Statistical Analyses

The data were analyzed by using windows software packages (spss), data were offered as the mean, ±standard deviation and ±standard error (SE). Statistical analysis of variance to compare between treated and control groups were tested by one way ANOVA (F-test). a level of statistically significant determination by P-value < 0.05.

Results

Changes in Some Types of Mature Peripheral Blood Cells

The results of numbers of (RBC, WBC and Thrombocytes) in control and experimental animals are shown in Table 1. Rabbits exposed to Azathioprine (group 2) showed a significant (P<0.05) decreased in numbers of (RBC, WBC and Thrombocytes) the mean and standard deviation were (6.323 ± 0.271, 5.159 ± 0.122, 314 ± 0.266) respectively, as compared to the control (group 2). In this context we see the results of the research) 3.976 ± 0.114, 2.994 ± 0.232, 147 ± 0.077) when comparing (group 3) with the control group (group 1) we observe a slight increase due to treatment with the extract but not a significant (P<0.05). While there was an almost a significant (P<0.05) clear rise in (group 5) (4.564 ± 0.154, 3.622 ± 0.241,182 ± 0.189) and (group 4) (4.895 ± 0.100, 3.933 ± 0.222,201 ± 0.103) when compared with control (group 2).

Results of the effect of the extracts in group 6, group 7, and group 8 showed a clear effect in raising the levels of the criteria under study. The study showed the strongest effect of the extract in (group 6) (5.737 ± 0.341, 4.897 ± 0.254,277 ± 0.351), (group7) (5.108 ± 0.222, 4.137 ± 0.206,226 ± 0.262) and (group8) (5.423 ± 0.111, 4.288 ± 0.244,253 ± 0.185) as compared to the control (group 2).

Table 1: Changes in some types of mature peripheral blood cells

Groups	RBC (millions/mm3) mean± SD	WBC (thousand/mm3) mean± SD	Thrombocytes (thousand/mm3) Mean ±SD
Group (1)	6.323 ± 0.271	5.159 ± 0.122	314 ± 0.266
Group (2)	3.154 ± 0.182	2.416 ± 0.103	108 ± 0.043
Group (3)	3.976 ± 0.114	2.994 ± 0.232	147 ± 0.077
Group (4)	4.895 ± 0.100	3.933 ± 0.222	201 ± 0.103
Group (5)	4.564 ± 0.154	3.622 ± 0.241	182 ± 0.189
Group (6)	5.737 ± 0.341	4.897 ± 0.254	277 ± 0.351
Group (7)	5.108 ± 0.222	4.137 ± 0.206	226 ± 0.262
Group (8)	5.423 ± 0.111	4.288 ± 0.244	253 ± 0.185

Changes in Some Types of Immature Peripheral Blood Cells

Our study results for each of the cells (IRF, IPF and IGs) were shown the results of the analyzes showed a different effect as follows (Group2) (3.154 ± 0.182,2.416 ± 0.103,108 ± 0.043); (Group 3) (3.976 ± 0.114,2.994 ± 0.232,147 ± 0.077); (Group 4) (4.895 ± 0.100,3.933 ± 0.222,201 ± 0.103); (Group 5) (4.564 ± 0.154,3.622 ± 0.241,182 ± 0.189); (Group 6) (5.737 ± 0.341,4.897 ± 0.254,277 ± 0.351); (Group 7) (5.108 ± 0.222,4.137 ± 0.206,226 ± 0.262);(Group 8) (5.423 ± 0.111, 4.288 ± 0.244,253 ± 0.185) as compared to the control (group 1) (4.4 ±0.247, 3.5± 0.911, 4.2±0.087).In this context, it emerged that the extract of (Group 6) was the most influential and the least effective was extracted in (Group 3). This effect is evident in the apparent decrease compared to control (group 2) where the group was given Azathioprine.

Table 2: Changes in some types of immature peripheral blood cells

Groups	IRF (%) ±SD	IPF (%) ±SD	IGs (%) ±SD
Group (1)	4.4 ± 0.247	3.5 ± 0.911	4.2 ± 0.087
Group (2)	43.2 ± 0.556	44.2 ± 0.011	1.7 ± 0.331
Group (3)	38.7 ± 0.111	33.4 ± 0.055	1.1 ± 0.081
Group (4)	26.2 ± 0.562	28.1± 0.097	2.5 ± 0.038
Group (5)	31.4 ± 0.227	30.3± 0.043	2.1± 0.026
Group (6)	12.4 ± 0.761	9.1 ± 0.097	3.6 ± 0.044
Group (7)	22.2 ± 0.752	25.2 ± 0.096	2.8± 0.012
Group (8)	19.1 ± 0.127	18.4 ± 0.015	3.3 ± 0.052

Change in Some Types of Peripheral Blood Cells

The final set of results was evaluated the following (Schistocytes, reticulocyte, ANC) Data on measurements in Azathioprine treated rabbits showed a marked increase a significant (P<0.05) in the number of cells examined in (group 2) (3.5 ± 0.098, 0.3± 0.247, 0.632 ± 0.021) all the extracts used in the study had different effects in scale down cell counts in table 3. As follows (Group 3) and (Group 5) groups (2.8 ±0.088,0.8 ± 0.055,1.237 ± 0.445); (2.3 ± 0.021,1.2 ± 0.021,1.544 ± 0.316) were less affected by the extract compared to other, (Groups 4) and (Groups 7) (1.2 ± 0.049,1.5± 0.029,1.843 ± 0.111); (1.4 ± 0.011,1.7 ± 0.045,2.212 ± 0.432)had a somewhat different effect, showing an average decrease a significant (P<0.05) from the rest of the extracts. (Groups 6) and (groups 8) (0.6 ± 0.022,2 ± 0.094, 3.261 ± 0.543); (0.8 ± 0.079,2 ± 0.067, 2.744 ± 0.331) had a large and clear effect a significant (P<0.05) and this was the effect of the increase especially in (group 6). All of these groups were compared to (group 2).

Table 3: Change in some types of peripheral blood cells

Groups	Schistocytes (%) ±SD	Reticulocyte (%) ±SD	ANC ±SD (thousand/mm3)
Group (1)	0.5 ± 0.054	2.1 ± 0.177	3.442 ± 0.323
Group (2)	3.5 ± 0.098	0.3± 0.247	0.632 ± 0.021
Group (3)	2.8 ± 0.088	0.8 ± 0.055	1.237 ± 0.445
Group (4)	1.2 ± 0.049	1.5± 0.029	1.843 ± 0.111
Group (5)	2.3 ± 0.021	1.2 ± 0.021	1.544 ± 0.316
Group (6)	0.6 ± 0.022	2 ± 0.094	3.261 ± 0.543
Group (7)	1.4 ± 0.011	1.7 ± 0.045	2.212 ± 0.432
Group (8)	0.8 ± 0.079	2 ± 0.067	2.744 ± 0.331

Discussion

It has become known that the current chemical treatments are very painful and have significant effects so many studies have been directed to try natural plant extracts as an alternative to chemical drugs, herbal drugs are the drugs that have plant origin. Some of the effective drugs that are used today for the treatment of major diseases from plants are isolated or are either structural modifications of compounds that are isolated from plants and plants and herbs are under research to treat pancytopenia are few [16]. Nigella sativa works to lower blood pressure, sugar level, cholesterol and also acts as anti-convulsive, anti-inflammatory and detoxifying agent in general [17].This plant consists of quinoid compounds, and has a high medical and pharmacological importance [18, 21].

This plant has been used in many forms of traditional medicine, proving its ability to treat blood pressure. In many cases it has been used as a protective compound to promote the metabolic activities of lung, liver and kidney; skin infections, many diseases of the digestive system. It is known for its wide effect in the treatment of bacterial diseases and anti-inflammatory and anti-fever. Several recent studies have shown that thymoquinone, a compound of this plant, regulates the cellular and humoral immune response, cellular infections and is used for its anticancer activity [22, 24].Medicago sativa, this plant is known as the father of all foods (alfalfa), a perennial and herbaceous plant of the legume family and the first origin of this plant in Asia [25]. M. sativa has been reported to be beneficial in the treatment of hemorrhage, as a tonic after blood loss and during anemia [26]. Moreover, it is considered beneficial in blood clotting disorders, kidney disorders, appetite stimulation, inflammation, increasing breast milk, indigestion, jaundice, increasing excretion of neutral steroids nutritional support, stomach thrombocytopenic purpura, vitamin supplementation (vitamins A, C, E, K) and wound healing [27].

North American Indians recommended alfalfa to treat jaundice and to encourage blood clotting [28] also Alfalfa is high in iron and powerful blood builders [29].Rocket seed meal (Eruca sativa) locally known as jarjeer contain vitamin C, carotenoids, flavonoids such as luteolin, appin and glucosinolates the precursors of sulfaraphene and isothiocyanates also contain volatile oils like apiola,-Bphellandrene and myristicin.

And it has several biological activities including antioxidant action, anticarcinogenic, anti-fungal, and anti-bacterial. It's known as antiinflammatory, diuretic and affects on content blood circulation. Eruca seeds have high level of oil, erucic acid and protein glucosinolate contents and commonly used as diet in animal feed in region of Asia, particularly in Pakistan and India [30, 33].

Other researcher said that this plant one of its components can control the anemia and also can affect of thrombocytopenia whereas affects the bone marrow and and the subsequent correction of the pathway of blood formation [34]. One of the researchers said in an experiment conducted on rabbits where animals were given many fresh plants, including Eruca sativa plant, where the researcher proved that Blood parameters returned to normal compared with control group [21, 35].

Mentioned that It is a Eruca sativa seeds plant contains many minerals, including iron, which is necessary for the formation of hemoglobin and therefore fights anemia [36].When using hyperglycemic rat model, said Ocimum basilicum seed extract improved the levels of many measurements, including AST, ALT, ALP, total bilirubin and total protein, serum electrolytes like Na+, K+, Cl-, HCO3-, haematological indices like red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), lymphocytes, neutrophils, eosinophils, monocytes and basophils. In a study that included the use of basil extract to try thalassemia and sickle cell anemia in children it was found that this extract has the ability to regroup the good hemochlobin[37].

In addition, this plant has the ability to remove free radicals as an antioxidant and also proved its ability to maintain normal body weight and where the effect of the extract in the return of some blood parameters to the natural level such as The numbers of WBC, platelet, and RBC, the percentages of neutrophils and monocytes, and the levels of Hb, PCV, MCV, MCH, and MCHC in wistar induced by phenylhydrazine for hemolytic anemia [38]. In a study conducted on type 2 diabetic patients, the patients were given Vitis vinifera seed extract. The research found that the seed extract acts as an antioxidant and thus improves the ability of liver enzymes which may cause improvement in blood standards[39].The researchers found that Vitis vinifera seed extract acts as an antibody to blood analysis. It was found during their research when the effect of radiation on the blood was tested. It was found that the seed extract protected the cell membrane from radiation damage [40]. In a research conducted on a number of donors that included the use of procyanidins from Vitis vinifera extract, which appeared from the results of the research of the levels of alpha-tocopherol in red blood cell membranes increased significantly and the lymphocyte oxidized DNA deoxyguanosine ratio was reduced and the red blood cell membrane faty acid composition shifted to a higher level of polyunsaturated fatty acids [41].

A study on the efficacy of Portulaca oleracea extract showed that the extract inhibited high-fat-diet-induced oxidative injury in a dose-dependent manner and proved it through a markedly dose-dependent from this extract reduction blood and liver lipid peroxidation levels and enhance antioxidant enzyme activities in HF mice [42,43].

The alcohol extract of this plant proved its ability to improve other metabolic aberrations[44].In a research conducted on many of the exhausted girls suffering from iron deficiency anemia, the study included the use of the extract of the seeds of this plant proved that this study has a positive effect increase in hemoglobin Hb hematocrit (Hct), and mean corpuscular volume and serum ferritin levels [45]. The crude extract of this plant has been proved by many useful reports on hematological profile and blood chemistry of rats, therefore, the study was carried out on a group of rats in order to measure the effect of this plant extract on erythrocyte osmotic fragility and which showed its high ability to reduce the fragility of blood cells[46].

Conclusion

To discover a way to replace the usual chemical drugs used in the treatment of pancytopenia because they have side effects, Plant extracts previously mentioned in this research were used as a trial alternative to commercial drugs, The Ocimum basilicum extract showed a higher effect than the rest of the extracts in pancytopenia treatment, which gave a clear effect on improving bone marrow efficiency, to confirm the effective role of these extracts in the treatment, there

must be many more thorough research on the tissue level of the bone marrow and the physiological level.

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